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of Experimental Stress Fractures

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REVISED REPORT

DAMD 17-98-1-8515

Specific responses:

The following is a revised report, written in response to reviewers' comments on our original report. The key comments/changes in this report are as follows:

1) The review raised question about "Contractual issues," specifically about the move of the original PI, Dr. Schaffler, to Mount Sinai Medical Center in New York, and the designation of Dr. Robert Boyd as the Co-PI to oversee the project in Michigan.

This changes was negotiated the contracting office in 1998.

2) There was a concern expressed about progress delays in the research.

We acknowledged in the original report for Year 2, as well as in our report from the previous year of the grant (Year 1), that we were behind schedule for the project. This results primarily from delays in Year 1 caused by a physical plant problem at the Henry Ford animal facility, which resulted in approximately 6 months delay in the start of work on this project. It should also be noted that because all of the loading experiments take several weeks to perform, and rely on one key piece of equipment, we will not be able to readily remediate these delays.

We also note that we have had to deal with turnover in key technical personnel, which further slowed progress.

3) The was a concern expressed about the lack of detail reported for our bone scan procedures, including details about normalization in accounting for soft tissue.

In our revised report, we now include details of the technetium bone scan procedures, including timing, sampling and normalization.

INTRODUCTION

Stress fractures result from repetitive loading and have been regarded as a mechanical fatigue-driven process. However, histopathological data and experimental data from our laboratory suggests that increased remodeling precedes the occurrence of bone microdamage and stress fractures, suggesting a central role for increased intracortical remodeling in the pathogenesis of stress fractures. Thus, we propose that stress fracture occurs through a positive feedback mechanism, in which increased mechanical usage stimulates focal bone turnover, resulting in a local increase in porosity. Microdamage accumulation and stress fractures result from continued cyclic loading of this transiently osteoporotic bone. These experiments test the hypothesis by pharmacologically inhibiting the bone remodeling response; the subsequent accumulation of microdamage and the severity of the stress fracture can be diminished. This hypothesis is being tested experimentally in the rabbit tibial stress fracture model, which was developed in our laboratory. To test the hypothesis that reactive remodeling within the cortex

drives the development of stress fractures, the effect of remodeling suppression using a bisphosphonate on the accumulation of bone microdamage and diminishing the severity of stress fracture will be examined. Outcomes of these experiments will be assessed using bone scintigraphy, histomorphometry, and biomechanical approaches.

SUMMARY OF RESEARCH

Our objectives in these experiments are to use the rabbit tibial stress fracture model: (1) to determine at the whole bone level whether bisphosphonate inhibition of intracortical remodeling attenuates the increase in focal bone ^{99m}Technetium uptake which characterizes the development of stress fracture, (2) to determine at the tissue level whether bisphosphonate inhibition of intracortical remodeling decreases the accumulation of cortical bone microdamage which occurs at the site of stress fracture, and (3) to determine how stress fracture compromises mechanical properties of long bones and whether pharmacological inhibition of remodeling can offset that functional deficit.

The project is proceeding toward the goals originally outlined for Year 2, with all procedures continuing. With the assistance of the Bioresources Department to permit the overlap of animal orders and the careful planning and scheduling of experiments, we were able to bring the progress of the mechanical loading of the rabbit hindlimbs closer into in line with expected goals.

We are behind schedule for initiating the histological analyses of stress fracture tibiae, due to staff turnover. Specifically, our histology technician left the Henry Ford Hospital in December 1999 and we have been unable to recruit a qualified candidate to replace her due to the very tight job market in this area. The position is posted and we continue to search for the appropriate person to fill this position. This has impacted our ability to process the histological material as we originally outlined in the goals for Year 2.

We have implemented an alternative plan for completing the tissue analyses. The tissues that are now archived and awaiting analyses will be sent to Dr. Schaffler's laboratory, at the Mount Sinai Medical Center in New York. There they will be processed and subjected to histomorphometric analysis, as per our original protocol

Results to date:

Bone scan procedure:

We have developed a standardized the procedure for ^{99m}Tc Technetium injection, scanning, and quantification to control for variability between animals and among groups. The animals were each injected with 3 mCurie of ^{99m}Tc starting at 2:00 PM in a predetermined sequence. The isotope was administered IV, with an injection time of about 5-6 minutes per rabbit. Scans were conducted 3 hours later to image the bone phase of ^{99m}Tc Technetium.

The rabbits are scanned using a General Electric STARCAM System with a pinhole collimator and the data archived on optical disk for later analysis. Prior to scanning, the rabbits are anesthetized with ketamine -xylazine and the lower extremities are placed into one of two positioning devices. The anterior positioning device captures the lower leg at the distal tibia and holds the legs with the anterior aspect of the leg toward the collimator. The lower limbs are slightly separated, parallel, and level. The positioning for the medial view places the lower extremity of the animal in a device that positions the legs at a 60° angle and level to the collimator. The total time to obtain both A-P and M-L images of each rabbit is about 12-15 minutes.

A standardized area is used to determine a region of interest at the stress fracture site for the anterior view. This region of interest has the same dimensions for all animals and provides an average count per pixel of isotope incorporation within the standard area. The same standardized area is also used to determine a background level of isotope incorporation. This region is distal to the site of stress fracture and also has the same dimensions for all animals. An average count of isotope incorporation per pixel is obtained within the background area. The average counts per pixel in the stress fracture region of interest are normalized by dividing by the average counts per pixel in the background region (Average Counts per Pixel Stress Fracture Region of Interest/Average Counts per Pixel Background Region). The mean value for each time period is compared between the bisphosphonate-injected and saline-injected groups.

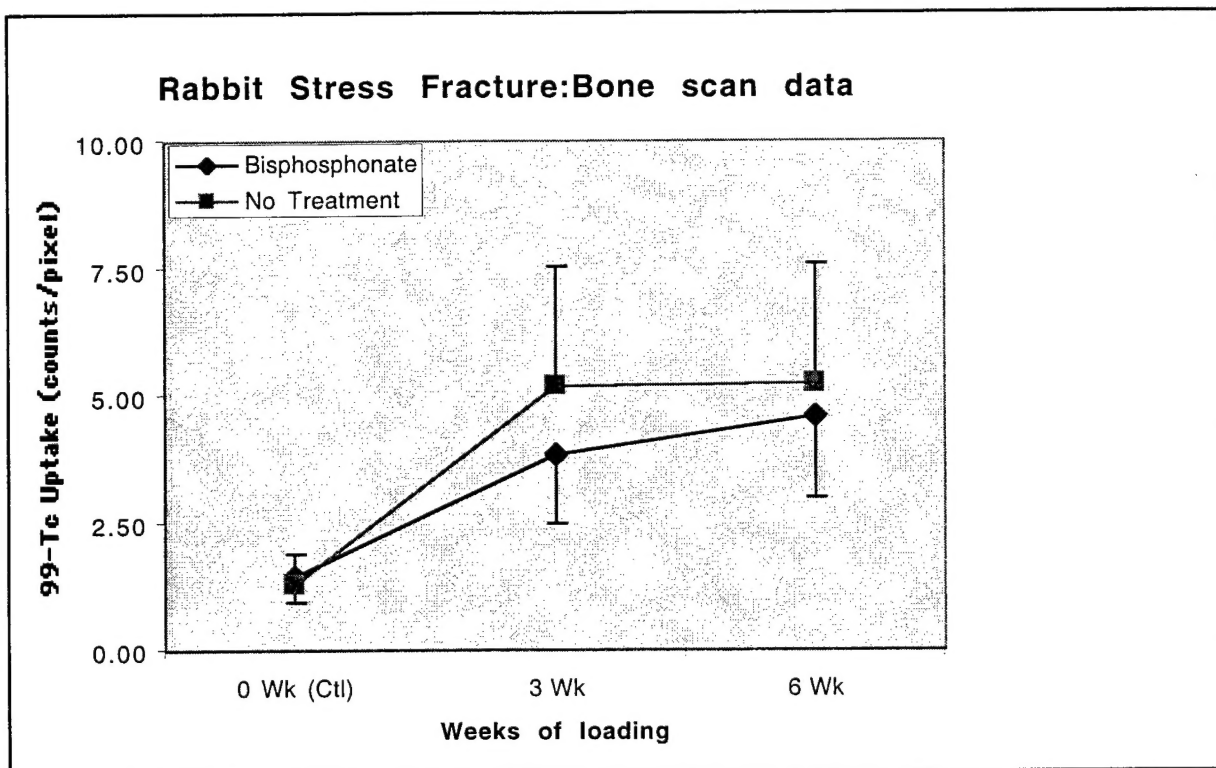
Results:

The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the stress fracture response, as indicated by technetium bone scans. This effect was most pronounced with short-term loading. Based on analyses of the experiments to date, bisphosphonate treatment produces a small reduction in technetium uptake relative to vehicle treated controls at all loading time periods (treated vs. control 26 percent lower at 3 weeks of loading ($p < .07$) and 13 percent lower after 6 weeks of loading ($p = \text{n.s.}$), Mann-Whitney U-test). These data are summarized in Figure 1.

Reduction of bone technetium uptake at 3 weeks in drug-treated animals is consistent with a suppression of the activation of new intracortical resorption foci by bisphosphonates. It is

unclear at this time why technetium uptake in bisphosphonate-treated animal at 6 weeks increases to control levels. Both an "escape" from remodeling suppression, as well as periosteal reaction resulting from long-term loading, could both account for this finding. Resolution of this of tissue mechanism question awaits completion of the histological studies.

Figure 1



YEAR 2: Goals:

The goals of the second year of the project were to continue to mechanically load rabbit hind limbs (with and without pharmacological inhibition of remodeling) on 32 rabbits (16 – 3 week duration experiments and 16 – 6 week duration experiments) to complete mechanical loading experiments for bone scans and histomorphometry analyses.

- Finish nonloaded controls (N=16 animals)
- Perform 64^{99m}Tc bone scans on loaded animals
- Analyze bone scans
- Harvest Tissues from these experiments
- Complete histological processing
- Begin histomorphometric analyses of loaded tibiae.

KEY RESEARCH ACCOMPLISHMENTS: YEAR 2

Key finding: The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the stress fracture response, as indicated by technetium bone scans. This effect was most pronounced with short-term loading.

Tasks accomplished specific to the grant Statement of Work:

- Mechanical loading completed on 48 experimental animals, distributed equally between bisphosphonate and vehicle treated animals
- 86^{99m}Tc bone scans and analyses were carried out on experimental and control animals to date
- All bone scans performed are analyzed
- All tissue from loaded animals has been harvested
- Histomorphometric analyses delayed due to loss of technician.

Note: *Our progress and accomplishments are revised from those originally projected in our statement of work.*

We are significantly behind our anticipated goals for this period. This results primarily from delays in Year 1 caused by a physical plant problem at the Henry Ford animal facility, which resulted in more than 6 months delay in the start of work on this project. These delays were detailed in our Year 1 progress report. Because experimental loading take several weeks to complete and relies on one key piece of equipment, this initial delay will propagate through the project, even as attempt to we catch up.

With the assistance of our Bioresources Department to permit the overlap of animal orders and the careful planning and scheduling of experiments, we were able to bring the progress of the mechanical loading of the rabbit hindlimbs closer into in line with expected goals. However, we remain behind schedule for the entire project for the reasons described

above. To date we now have completed mechanical loading and bone scan studies on 48 experimental animals, and have completed 16 non-loaded controls.

We also note that we have had to deal with turnover in key technical personnel, which has further slowed our progress.

REPORTABLE OUTCOMES

None to date. Experiments are ongoing.

CONCLUSIONS

None to date. Experiments are ongoing.